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COMBINED EFFICACY OF ASCORBIC ACID AND METHYLENE BLUE IN EXPERIMENTAL METHAEMOGLOBINAEMIA IN BUFFALO CALVES WITH SUB-CLINICAL HEPATITIS.

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ABSTRACT

In five male buffalo calves with sub-clinical hepatitis, methaemoglobinaemia was induced by intraruminal administration of sodium nitrite (BDH) @ 100 mg/kg b.wt. After 1 hr. ascorbic acid @ 15 mg/kg b.wt. and methylene blue @ 5 mg/kg b.wt. (1% aqueous solution) along with 500 ml dextrose (5%) were infused. The efficacy was judged based on clinical and blood biochemical response. Marked improvement was noted in clinical signs like tachycardia, polyurea, lacrimation and inappetence within 4 hr of treatment. The methaemoglobin per cent dropped dramatically by 4 hr. The haemoglohin/methaemoglobin ratio, blood glucose and serum sodium regained their normal levels and plasma became nitrite free by 8 hr. The Glutamic oxaloacetic transaminase (GOT) level normalised by 48 hr while the Bromsulphalein (BSP) T½ remained high even up to 72 hr. No marked change could be noted in Glutamic pyruvic transaminase (GPT) level.

Heavy application of nitrogen-rich fertilizers, weedicides and pesticides condition the cereal as well as fodder plants to accumulate excess nitrate in them. And on ingestion of such plants, ruminants suffer from nitrate/nitrite toxicosis. Ascorbic acid has been found to be effective in the management of experimental nitrite intoxication in buffalo calves (Prasad et al.,1984). However, the rate of reduction of methaemoglobin was slow as compared to methylene blue (Ziv et al., 1982). The action of methylene blue depends on the availability of a set of enzymes (Jones et al., 1977) and dosage above 10 mg/kg b.wt. forms additional methaemoglobin in calves (Dua & Prasad, 1985). In the present study, the rate of reduction of methaemoglobin jointly by ascorbic acid and methylene blue has been judged on the basis of clinico-haemochemical improvements in buffalo calves.

MATERIALS AND METHODS

Five clinically healthy buffalo calves, 1½ years old and weiging between 90 - 110 kg were used in the present investigation. They were screened for parasitic infestation. Carbontetrachloride (CCl_4) @ 0.3 ml/kg b.wt. was injected intraruminally. Bromosulphaphthalein (BSP) excretion test was applied 72 hr later to confirm hepatic damage. Following overnight fast, a bolus dose of sodium nitrite (BDH) @ 100 mg/kg b.wt. in about 150 ml water, was drenched with stomach tube intraruminally. Clinical observations and collection of blood samples (4-5 ml heparinised and 15 ml for serum) were made at 0,0.5,1,2,3,4,8, 24,48 and 72 hr. In all the animals a single dose of ascorbic acid @ 15 mg/kg b.wt. and methylene blue 5 mg/kg b.wt. (1%) aqueous solution) along with 500 ml dextrose (5%) was infused intravenously one hour postnitrite drench. A course of Livogen 5 ml (i/m) was given daily for rapid regeneration of liver.

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Blood samples were processed for haemoglobin and methaemoglobin (Raymond and Wilkinson, 1969), blood glucose (Frankel et al.1970), serum glutamic oxaloacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) (Reitman and Frankel, 1957), ammonia–N (Conway, 1957), sodium and potassium (Oser, 1971), plasma nitrite (Evans and Nason,1953), and BSP T½ and fractional clearance (Kaneko, 1980). The experimental results were compared with the O hr values. The observations to assess liver damage were compared with the values on control animals (Prasad,1983).

RESULTS AND DISCUSSION

Following one hr of nitrite drench, all the animals developed dullness, disorientation, polyurea and inappetence. These clinical symptoms improved by 4 hr of treatment, Chocolate brown conjunctival mucous membrane and episcleral blood vessels returned pinkish in appearance at the same time, indicating a rapid reduction of methaemoglobin. It took 24 hr for the conjunctival mucous membrane of animals treated with ascorbic acid alone to become normal (Prasad et al. 1984). The accelerated heart rate normalised by 3 hr of treatment and the respiratory rate by 24 hr.

Table 1 Haemo – biochemical response to ascorbic acid and methylene blue treatment in experimental Methaemoglobinaemia in buffalo calves n–5 (Mean ±(SE)

	Interval Hr (s)									
Parameters	0	0.5	1	2	3	4	8	24	48	72
Haemoglobin (g%)	11.20 ±0.14	10.84 ±0.15	10.48 ±0.34	10.32 ±0.41	10.48 ±0.52	10.40 ±0.45	10.72 ±0.47	10.76 ±0.30	10.84 ±0.21	10.92 ±0.20
Methaemoglobin (%)	6.02 ±0.66	27.82★ ±0.32	54.94★ ±1.74	26.30★ ±0.26	15.72★ ±2.43	10.26 ±1.91	7.66 ±0.74	5.98 ±0.35	5.98 ±0.33	5.30 ±0.27
Haemoglobin/ Methaemoglobin ratio	18.26 ±3.01	3.75★ ±0.35	1.83★ ±0.05	4.07★ ±0.56	7.59★ ±2.02	12.75 ±3.07	15.00 ±2.87	17.00 ±1.19	17.21 ±0.93	19.09 ±1.04
Blood Glucose (mg%)	62.32 ±3.86	67.68 ±4.69	84.00 ±7.11	86.12★★ ±6.25	83.90★★ ±5.90	80.08 ±6.62	74.06 ±6.85	64.54 ±7.75	60.84 ±4.17	59.84 ±2.75
Serum Ammonia (mg%)	0.61 ±0.12	0.63 ±0.41	0.67 ±0.08	0.81 ±0.11	0.85 ±0.08	0.68 ±0.14	0.81 ±0.14	0.60 ±0.09	0.61 ±0.09	0.60 ±0.08
SGOT (Units/ml)	49.60★ ±5.19	50.80★ ±3.47	54.40★ ±0.66	52.80★ ±5.04	50.60★ 5.19	53.00★ ±6.08	55.00★ ±4.23	37.60★★ ±3.54	25.20 ±1.20	19.60 ±2.13
SGPT (Units/ml)	8.40 ±2.04	9.60 ±2.40	10.40 ±2.04	10.00 ±2.19	9.20 ±1.09	11.60 ±2.23	12.50 ±2.11	9.60 ±2.40	8.80 ±2.40	8.80 ±1.20
Serum Sodium (mEg/L)	160.80 ±2.06	166.80 ±1.62	175.60 ±2.48	176.80★ ±4.02	177.80★ ±2.76	175.80★ ±8.47	168.20 ±5.58	159.80 ±2.15	159.00 ±2.09	158.00 ±1.90
Serum Potassium (mEg/L)	6.24 ±0.18	6.86 ±0.31	7.52★ ±0.21	7.52★ ±0.16	7.44★ ±0.11	6.68★ ±0.09	7.26★ ±0.19	6.46 ±0.09	6.16 ±0.23	6.04 ±0.17
Plasma Nitrite (mcg%)	NT	90.00 ±19.31	238.00 ±22.22	148.00 ±26.15	24.90 ±11.13	17.90 ±8.00	NT	NT	NT	NT
BSP T½	11.38 ±0.65	–	–	–	–	–	11.52 ±0.74	9.88 ±6.65	8.06 ±0.56	6.74 ±0.39
Fractional Clearance (K%)	0.062 ±0.003	–	–	–	–	–	0.061 ±0.001	0.071 ±0.005	0.087 ±0.006	0.104 ±0.005

★ Statistically significant at 1% level ($P < .01$) NT : Not traceable

★★ Statistically significant at 5% level ($P < .05$)

The body temperature did not vary much throughout the experiment.

A marginal lowering of haemoglobin could be noted initially but subsequently it varied little (Table 1) owing to prompt relief from anoxic stress. The methaemoglobin level rose to 54.94 ± 1.74 at one hr, returned to 10.26 ± 1.91 per cent at 4 hours of therapy, indicating more rapid reduction of methaemoglobin jointly by ascorbic acid and methylene blue than by ascorbic acid alone (Prasad et al. 1984). Dramatic decline of methaemoglobin, from 55–60 to less than 20 per cent, within a period of 0.5–2 hr of methylene blue infusion (@ 10 mg/kg b.wt.) in steers suffering from nitrite toxicosis has been noted (Ziv et al. 1982). The haemoglobin/methaemoglobin ratio, which was 1.83 ± 0.85 at one hr, regained its normal level by 4 hr of therapy. All the five calves survived owing to rapid reduction of methaemoglobin jointly by ascorbic acid and methylene blue.

Initially, hyperglycaemia was noted, which normalised by 4 hr of treatment. Again, the response was the outcome of relief of methaemoglobinaemic stress. Marginally raised level of serum ammonia came to normal value by 24 hr. The concentration of SGOT remained elevated up to 24 hr, suggesting hepatic cell damage. The level came to pre–CCl_4 administration by 72 hr, indicating arrest of hepatic cell damage. Serum SGPT varied little throughout the experiment. Hypernatraemia and hyper–kalaemia were recorded at the start of treatment. The level of sodium normalised by 8 hr and that of potassium by 24 hr. From a non–detectable level, the plasma nitrite was 238 ± 22.23 mcg. around one hr after nitrite drench. Nitrite–free plasma within 8 hr indicated its quick elimination. The short elimination half time (47.50 min) of nitrite in sheep supported its rapid removal from plasma (Schneider & Yeary, 1975). All the animals had increased BSP T½ and decreased fractional clearance even up to 72 hr of the experiment. Normalization of SGOT level at 72 hr (vide supra) indicated arrest of cellular damage while increased BSP T½ and decreased fractional clearance suggested structural deformity in the liver (Prasad et al.,1982).

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APPLICATION OF ENZYME

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good colour reaction. Rai and Lahire (1981) got similar results after keeping coated plates overnight at 4°C . However, Abu Elzein and Crowther (1978) obtained better results by keeping the plates at room temperature for 2 hours.

Information on the use of ELISA in the study and estimation of FMD antibodies in buffalo sera is lacking. The indirect ELISA could be used to estimate the level of antibodies in buffalo sera and this offered a good tool for the study of immunity against FMD virus vaccine. In the field of non-isotopic immunoassays, ELISA is the most repidly growing segment. Drawbacks associated with the routine clinical use of radioactive reagents could be avoided easily. Moreover, simplicity of protocol, availability of suitable instrumentation, less reagent cost and time make ELISA a favourable tool.

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